A red light-controlled synthetic gene expression switch for plant systems

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Supplementary Table 1 | Expression vectors and oligonucleotides designed and used in this study.
Supplementary Figure 1 | Effect of clarithromycin on constitutive gene expression in N. tabacum
Supplementary Figure 2 | Spectrum of the white light source
Supplementary Table 1

Expression vectors and oligonucleotides designed and used in this study.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Description</th>
<th>Ref. or source</th>
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<tbody>
<tr>
<td>auxin</td>
<td>Plasmid encoding P&lt;sub&gt;CamV35S&lt;/sub&gt; controlled optimized auxin sensor (P&lt;sub&gt;CamV35S&lt;/sub&gt;-RLuc-p2A-SM[L2min17]-Fluc-pA)</td>
<td>1</td>
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<tr>
<td>pKM002</td>
<td>Vector encoding SEAP under control of a modified P&lt;sub&gt;Tet&lt;/sub&gt; (tetO&lt;sub&gt;13&lt;/sub&gt;-394bp-P&lt;sub&gt;hCMVmin&lt;/sub&gt;-SEAP-pA)</td>
<td>2</td>
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<tr>
<td>pKM006</td>
<td>Vector encoding SEAP under the control of a modified P&lt;sub&gt;Tet&lt;/sub&gt; (tetO&lt;sub&gt;13&lt;/sub&gt;-422bp-P&lt;sub&gt;hCMVmin&lt;/sub&gt;-SEAP-pA)</td>
<td>2</td>
</tr>
<tr>
<td>pKM022</td>
<td>Bicistronic vector encoding PhyB(1-650)-VP16-NLS and Tet-R-PiF6(1-100)-HA under control of P&lt;sub&gt;SV40&lt;/sub&gt; (P&lt;sub&gt;SV40&lt;/sub&gt;-PhyB(1-650)-VP16-NLS-IRE&lt;sub&gt;Py&lt;/sub&gt;-Tet-R-PiF6(1-100)-HA-pA)</td>
<td>2</td>
</tr>
<tr>
<td>pKM033</td>
<td>Vector encoding VEGF&lt;sub&gt;121&lt;/sub&gt; under control of a modified P&lt;sub&gt;Tet&lt;/sub&gt; (tetO&lt;sub&gt;13&lt;/sub&gt;-422bp-P&lt;sub&gt;hCMVmin&lt;/sub&gt;-VEGF&lt;sub&gt;121&lt;/sub&gt;-pA)</td>
<td>2</td>
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<tr>
<td>pKM081</td>
<td>Vector encoding SEAP under control of a modified P&lt;sub&gt;ETR&lt;/sub&gt; (etra&lt;sub&gt;13&lt;/sub&gt;-P&lt;sub&gt;hCMVmin&lt;/sub&gt;-SEAP-pA)</td>
<td>3</td>
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<tr>
<td>pKM082</td>
<td>Vector encoding SEAP under control of a modified P&lt;sub&gt;ETR&lt;/sub&gt; (etra&lt;sub&gt;2&lt;/sub&gt;-386bp-P&lt;sub&gt;hCMVmin&lt;/sub&gt;-SEAP-pA)</td>
<td>This work</td>
</tr>
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</table>

A 372 bp fragment was amplified from CFP using oligos oKM090 (5'-caagtagctagcCCCTGAAATTCTGCACC-3') and oKM003 (5'-caagtagctagcTCTTGAAGTTGGCCCTTGGATGC-3'), digested (NheI) and ligated (NheI) into pKM081.

pKM271                  | Vector for P<sub>CamV35S</sub>-controlled expression of PiP-VP16-NLS (P<sub>CamV35S</sub>-PiP-VP16-NLS-pA) | This work      |

PiP-VP16-NLS was amplified from pMF156 using oligos oKM373 (5'-caagtagctagcctatgcggtggcATGAGTCGAGGAGAGGGTCG-3') and oKM374 (5'-caagtagctagccacctccctctcttgcgCCACCGTACTCGTCAATCC-3'), digested (Ndel/EcoRI) and ligated (Ndel/EcoRI) into pM2824.

pKM272                  | Vector encoding Fluc under control of a modified P<sub>Ptr</sub> (P<sub>Ptr</sub>-3-P<sub>hSP70min</sub>-Fluc-pA) | This work      |

Fluc was excised (EcoRI/HindIII) from pM2836 and ligated (EcoRI/HindIII) into pMF199.

pKM295                  | Vector encoding VEGF under control of a modified P<sub>ETR</sub> (etra<sub>1</sub>-P<sub>hCMVmin</sub>-VEGF<sub>121</sub>-pA) | This work      |

VEGF<sub>121</sub> was excised (EcoRI/NotI) from pKM033 and ligated (EcoRI/NotI) into pM2836.

pKM300                  | Bicistronic vector encoding PhyB(1-650)-VP16-NLS and E-PiF6(1-100)-HA under control of P<sub>SV40</sub> (P<sub>SV40</sub>-PhyB(1-650)-VP16-NLS-IRE<sub>Py</sub>-E-PiF6(1-100)-HA-pA) | This work      |

IRES<sub>Py</sub> was amplified from pKM022 using oligos oKM400 (5'-ACCCACCCCAGAGGCCC-3'), while P<sub>SV40</sub> was amplified from pKM033 (5'-caagtagctagcTCTTGAAGTTGGCCCTTGGATGC-3'), digested (NheI) and ligated (NheI) into pKM081.

pKM301                  | Bicistronic vector encoding PhyB(1-650)-VP16-NLS and PiP-VP16(1-100)-HA under control of P<sub>SV40</sub> (P<sub>SV40</sub>-PhyB(1-650)-VP16-NLS-IRE<sub>Py</sub>-PiP-VP16(1-100)-HA-pA) | This work      |

IRES<sub>Py</sub> and PiP-VP16(1-100) were amplified as described for pKM300. PiP was amplified from pMF150 using oligos oKM406 (5'-atccagatggcgaatggcgtggcgtggcgtgCCACCATGAAATTCTGCACC-3') and oKM407 (5'-atccagatggcgaatggcgtggcgtggcgtgCCACCATGAAATTCTGCACC-3').

pMF150                  | Vector encoding PiP under control of P<sub>hCMV</sub> (P<sub>hCMV</sub>-PiP-pA) | 4              |

pMF156                  | Vector encoding PiP under control of P<sub>SV40</sub> (P<sub>SV40</sub>-PiP-VP16-pA) | 4              |

pMF199                  | Vector encoding SEAP under control of a modified P<sub>Ptr</sub> (P<sub>Ptr</sub>-3-P<sub>hSP70min</sub>-SEAP-pA) | 4              |

pMK052                  | Vector encoding P<sub>Ptr</sub>-controlled TiR1 (P<sub>TiR1</sub>-TiR1-pA) | 5              |

pMZ202                  | Vector encoding Fluc under control of a modified P<sub>Tet</sub> (tetO<sub>13</sub>-P<sub>hCMVmin</sub>-Fluc-pA) | This work      |

Fluc was amplified from pSW209 using oligos oMZ207 (5'-atccataggctgtggcgtggcgtggcgtgCCACCATGAAATTCTGCACC-3') and oMZ208 (5'-atccataggctgtggcgtggcgtggcgtgCCACCATGAAATTCTGCACC-3').

pMZ224                  | Vector for P<sub>CamV35S</sub>-controlled expression of E-VP16-NLS (P<sub>CamV35S</sub>-E-VP16-NLS-pA) | E-VP16-NLS was amplified from pWW035 using oligos oMZ209 (5'-atccataggctgtggcgtggcgtggcgtgCCACCATGAAATTCTGCACC-3').

This work
gtgtaaacgccgtcgctgtgcgtttcctctctctttttgtccacccgctagcaaac-3') and oMZ827 (5'-
tgaacgccgtcgctgtgcgtttcctctctctttttgtccacccgctagcaaac-3'), while the backbone of
pSW209 was amplified using oligos oMZ873 (5'-gcaacggctagcacgacgtgacggtggtcagcgagtttc-3')
and oMZ874 (5'-gtgaacgccgtcgctgtgcgtttcctctctctttttgtccacccgctagcaaac-3'), while the
backbone of pSW209 was amplified using oligos oMZ873 and oMZ874. Finally, both fragments
were fused by Gibson cloning.

pMZ827 Vector encoding P\textsubscript{CAMV35S}-controlled nuclear-targeted E-PIF6 (1-100) (P\textsubscript{CAMV35S-E-PIF6(1-100)-NLS-pA})
E-PIF6 was amplified from pKM300 using oligos oMZ895 (5'-
tgaacgacgcatatgacaacaATGCCCCGCCCCAAGCTCAAG-3') and oMZ8127 (5'-
tcaacgtctgtgctagccgttgcctacaccttcctcttcttctttggGTCAACATGTTTATTGCTTTCCAACATGTTTG-

pMZ828 Vector encoding P\textsubscript{CAMV35S}-controlled nuclear-targeted E-PIF6 (1-100) (P\textsubscript{CAMV35S-E-PIF6(1-100)-NLS-pA})
E-PIF6 was amplified from pKM300 using oligos oMZ895 (5'-
tgaacgacgcatatgacaacaATGCCCCGCCCCAAGCTCAAG-3') and oMZ8127 (5'-
tcaacgtctgtgctagccgttgcctacaccttcctcttcttctttggGTCAACATGTTTATTGCTTTCCAACATGTTTG-

pMZ828 Vector encoding P\textsubscript{CAMV35S}-controlled nuclear-targeted PhyB (1-650)-VP16 (P\textsubscript{CAMV35S-PhyB(1-650)-VP16-NLS-pA})
PhyB-VP16-NLS was amplified from pKM300 using oligos oMZ856 (5'-
gccatggtgagcacgGTCGACTCTAGATCACACCTTCCG-3') and oMZ8123 (5'-
tcaacgtctgtgctagccgttgcctacaccttcctcttcttctttggCCCACCGTACTCGTCAATTCCAAG-3'), while the
backbone of pSW209 was amplified using oligos oMZ873 and oMZ874. Finally, both fragments
were fused by Gibson cloning.

pMZ833 Vector for P\textsubscript{CAMV35S}-controlled expression of TetR-VP16-NLS (P\textsubscript{CAMV35S-TetR-VP16-NLS-pA})
Tet-VP16 was amplified from pSAM200 using oligos oMZ891 (5'-
tgaacgacgcatatgacaacaCGGCCGCCACCATGTCTAGATTAG-3') and oMZ8123 (5'-
tcaacgtctgtgctagccgttgcctacaccttcctcttcttctttggCCCACCGTACTCGTCAATTCCAAG-

pMZ836 Vector encoding FLuc under control of a modified PETR (etr8-PhCMVmin-FLuc-pA)
FLuc was amplified from pSW209 using oligos oMZ807 and oMZ808, while the backbone of pKM081 was
amplified using oligos oMZ809 (5'-gtgtaaacccgtccatggcgtCGTGGTCCCCGCGTTGCTTC-3') and oMZ810 (5'-
cgccagcgcagccaattgagcGGAAGCTGACTCTAGAGGATCCCC-3'). Finally, both fragments were fused by
Gibson cloning.

pMZ837 Vector encoding P\textsubscript{CAMV35S}-driven expression of miRNA\textsubscript{TIR1} (P\textsubscript{CAMV35S-miRNA\textsubscript{TIR1}-pA})
For the design of an miRNA targeting N. tabacum TIR1 the online tool CentroidFold\textsuperscript{6} was used and miRNA
cloning was performed by a modification of a previously described protocol.\textsuperscript{7} First, the 5'-stem sequence
was amplified from pRS300 using oligos oMZ880 (5'-
tgaacgacgcatatgacaacaGAGGTCGACGGTATCGATAAGCTTG-3') and oMZ8163 (5'-
cggtagacaaattggatcattgattctctttggtggtcaactgactggattgtCTCTCTCTTTTGTATTCCAATTTTCTT-

pMZ839 Vector encoding miRNA\textsubscript{TIR1} under control of a modified \textsubscript{P}\textsubscript{ETR} (\textsubscript{P}\textsubscript{etra-P\textsubscript{CAMV35S-miRNA\textsubscript{TIR1}-pA})
miRNA\textsubscript{TIR1} was amplified from pmZ837 using oligos oMZ8118 (5'-
gccacgcggtgctgtgctggcggCCACCTGAACGACGCATATGACAAC-3') and oMZ8119 (5'-
cagctagctagtacctacatgccatctatgcgaattgattgtCCATCTATATATATCTTAAACATCAA-3'). Next,
both PCR-products were extended by overlapping loop sequences by amplification with oMZ880 and
oMZ882 (5'-
cgagtctagtttgaattttggcgactcggtatttggatgaatgagtcgGAAGCTAATTGAATCATATCACGACCTGTGAG-

pMZ841 Vector encoding TIR1 under control of a modified \textsubscript{P}\textsubscript{ETR} (\textsubscript{P}\textsubscript{etra-P\textsubscript{CAMV35S-miRNA\textsubscript{TIR1}-pA})
TIR1 was excised (EcoRI/XbaI) from pMK052 and ligated (EcoRI/SpeI) into pKM081.

pSW209 Vector encoding firefly luciferase and renilla luciferase separated by a 2A-peptide under control of
P\textsubscript{CAMV35S} (P\textsubscript{CAMV35S-FLuc-p2A-RLuc-pA}).

pWW035 Vector encoding P\textsubscript{SV40}-driven expression of E-VP16 (P\textsubscript{SV40-E-VP16-pA})

pWW043 Vector encoding P\textsubscript{SV40}-driven expression of E-KRAB (P\textsubscript{SV40-E-KRAB-pA})
E, macrolide-responsive repressor protein; etr, operator sequence binding E; FLuc, firefly luciferase; HA, human influenza hemagglutinin-derived epitope tag; IRES<sub>PV</sub>, polioviral internal ribosome entry site; KRAB, transcriptional repressor domain from human Kox1; NLS, nuclear localization signal from simian virus 40 large T antigen; pA, polyadenylation signal; p2A; foot-and-mouth disease virus-derived self-processing 2A peptide; P<sub>CaMV35S</sub>, cauliflower mosaic virus 35S promoter; PiP, pristinamycin-induced protein, P<sub>hCMV</sub>, human cytomegalovirus immediate early promoter; P<sub>hCMVmin</sub>, minimal human cytomegalovirus immediate early promoter; P<sub>HSP70min</sub>, minimal heat-shock protein 70 promoter from Drosophila; PhyB, Phytochrome B; PhyB(1-650), N-terminus of Phytochrome B with amino acids 1-650; PIF6, Phytochrome-interacting-factor 6; PIF6(1-100), N-terminus of Phytochrome-interacting-factor 6 with amino acids 1-100; P<sub>SV40</sub>, simian virus 40 early promoter; PIR, operator sequence binding PiP; P<sub>Tet</sub>, tetracycline-responsive promoter; RLuc, renilla luciferase; SEAP, human placental secreted alkaline phosphatase; SM, auxin sensor module; tetO, operator sequence binding TetR; TetR, tetracycline repressor protein; TIR1, auxin receptor transport inhibitor response 1; VEGF<sub>121</sub>, 121 amino acids splice variant of human vascular endothelial growth factor; VP16, <i>Herpes simplex</i> virus-derived transactivation domain.

Uppercase in oligos, annealing sequence; underlined sequence, restriction site.
**Supplementary Figure 1**

**Effect of clarithromycin on constitutive gene expression in *N. tabacum*.** 125,000 protoplasts were transformed for constitutive firefly luciferase expression. After incubation for 24 h in the absence (-AB) or presence of 100 µg ml⁻¹ clarithromycin, the firefly luciferase luminescence was quantified. Data are means ± SEM (n=12).
**Supplementary Figure 2**

*Spectrum of the white light source.* The light spectrum between 300 nm and 800 nm was recorded using an Avaspec-ULS2048 spectroradiometer (Avatec).


